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Structural Studies on the Ribbon-to-Helix Transition in Profilin : Actin Crystals

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ABSTRACT Knowledge of the structure of actin in its various conformational states is important for understanding the diverse motile activities carried out by eukaryotic cells. Profilin:actin crystals provide a unique system for studying conformational states of actin, because they exhibit a high degree of polymorphism in response to environmental conditions while maintaining crystalline order. A preliminary comparison of two states of profilin:β-actin crystals shows that crystal polymorphism involves movements of actin subdomains at hinge points homologous to those found in hexokinase, a protein whose polypeptide fold is related to actin. The homology of the hinge points in actin to those in hexokinase suggests that actin subdomain movements in profilin:β-actin crystals have functional significance. We discuss how these movements could be related to structural transitions between states of filamentous actin in muscle contraction.

INTRODUCTION

The actin powerstroke model of muscle contraction (Schutt and Lindberg, 1992, 1993) suggests that actin filaments (F-actin) can exist in two states: the classical helical form (H-actin) and a ribbon form (R-actin) brought about by a twist and a stretch of each monomer. Two distinct waves of transitions between these F-actin states, ribbonization (helix-to-ribbon) and helicalization (ribbon-to-helix), would travel along actin filaments as the muscle develops tension. At any given instant, forces developed independently in local segments along actin filaments are summed and transmitted to the ends of the sarcomere by tropomyosin filaments. We describe here the structural basis for this model.

PROFILIN:ACTIN RIBBONS

The crystal structure of bovine profilin:β-actin (Schutt et al., 1993) revealed an unusual organization of actin and profilin molecules. As shown in Fig. 1, each actin molecule makes an extensive contact with two other actin molecules across a 2_1-screw axis parallel to the *b* dimension of the unit cell. The ribbon contacts in this extended structure, which we have called the actin "ribbon," closely resemble the interactions between monomers in oligomeric assemblies of proteins (Schutt et al., 1993). The actin:actin interface across the ribbon axis buries a total of 1777 Å^2 of solvent-exposed surface area and uses interactions between close-packed hydrophobic side chains. Each profilin molecule intercalates between adjacent actin molecules along the ribbon axis. Ribbon contacts are contiguous, so that the end of one contact defines the beginning of the next. Thus, an unbroken contact forms a spine extending along the entire length of the ribbon.

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STRUCTURAL TRANSITIONS IN β-ACTIN

Profilin:actin crystals are unique in that they diffract to high resolution even as they undergo a reversible change in unit cell dimension *c* from 186.5 to 168.0 Å (Schutt et al., 1989). These unit cell transitions are observed in single crystals in response to such changes in solution characteristics as ionic strength, pH, temperature, nucleotide state, and presence of specific ions. Even more remarkable is that changes of nucleotide or profilin affinity transform dramatically the diffraction patterns of profilin:actin crystals; sharp, nonintegral, diffuse reflections appear, indicating the presence of a higher order perturbation of the basic ribbon motif that may be due to a pretransition to a fiber-like state (Schutt et al., 1989). Thus, profilin:actin crystals may hold the key to understanding structural transitions in actin and their relevance to chemomechanical transduction.

It is now possible to provide a molecular description of some of these transitions by comparing structures of profilin:β-actin in the "tight" and "expanded" states (Rozycki et al., 1995). Although the expanded-state structure is still being refined, it appears already to differ significantly from both the tight-state structure of β-actin (Schutt et al., 1993) and the structure of α-actin extracted from the DNase I:α-actin complex (Kabsch et al. 1990). These structures are compared in Fig. 2. The most obvious effect is a progressive opening of the cleft between the large and small domains, with α-actin having the smallest opening and β-actin in the expanded state having the largest.

THE RIBBON AS A STARTING POINT FOR MODELING f-ACTIN

The significance of the profilin:actin "ribbon" as a physiologically important structural entity has yet to be proven experimentally. However, we believe that the ribbon is structurally related to the helical actin filament (Schutt et al., 1989, 1993, 1994) for five reasons. First, profilin:actin crystal growth competes with F-actin assembly in vitro. As a

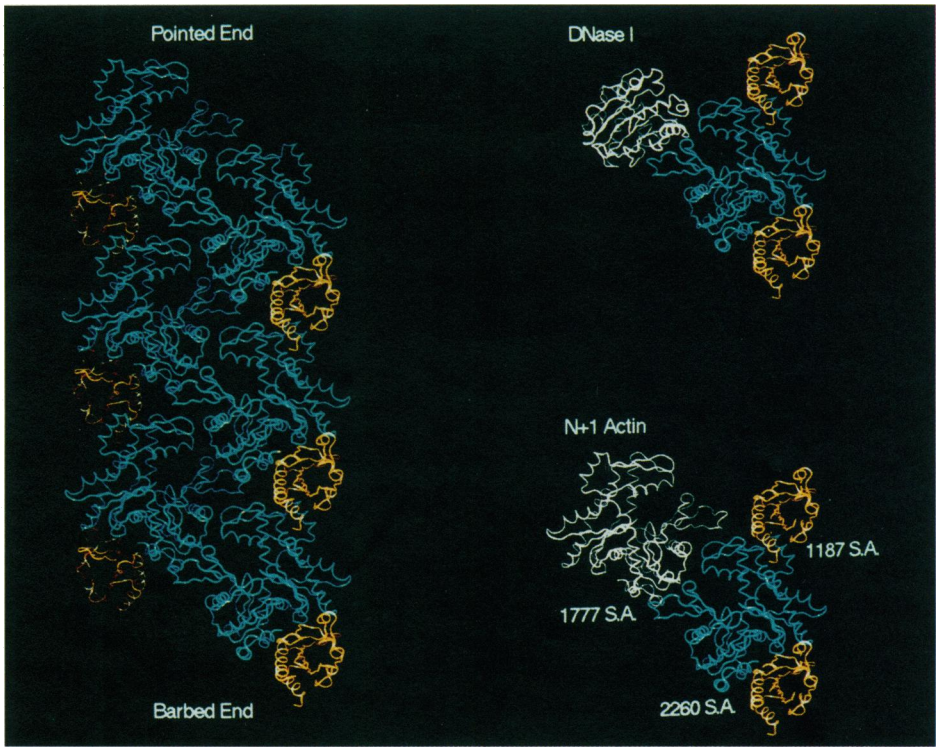

FIGURE 1 Structure of ribbons in crystalline profilin:β-actin (Schutt et al., 1993). (*left*) Actin molecules (*blue*) are organized about a 2_1-screw axis, oriented vertically in the figure. Profilin (*gold*) intercalates between adjacent actin molecules lying on the same side of the 2_1-screw axis. The "barbed end" and "pointed end" are defined by the binding site of gelsolin segment-1 to actin (McLaughlin et al., 1993). (*lower right*) A "ribbon contact" occurs at the interface of two actin molecules lying diagonally across the symmetry axis from each other. One of the actin molecules (N+1) is shown in white for contrast. Each actin molecule participates in two ribbon contacts, so that the end of one contact defines the beginning of the next. Each actin molecule also makes two extensive contacts with profilin. Profilin and actin lie approximately in a single plane on each side of the 2_1-screw axis. The buried solvent-accessible surface areas for the ribbon contact and the two profilin:actin contacts were calculated according to Lee and Richards (1971) with X-PLOR (Brünger, 1992), using a probe radius of 1.4 Å. The values given in the figure differ somewhat from those reported in Schutt et al. (1993) because of further refinement of the profilin:β-actin model. (*upper right*) The N+1 monomer in the lower panel docks to the same site in subdomains 2 and 4 of actin that is used in binding DNase I, an inhibitor of actin filament assembly. This figure was made by superimposing the DNase I:α-actin structure (Kabsch et al., 1990) onto β-actin, and then removing α-actin from the image.

result, actin may use related protein:protein contacts in both filaments and crystals, as is the case for sickle-cell hemoglobin (Rogers et al., 1986). Second, the actin:actin interface in the ribbon is extensive and displays characteristics of oligomeric protein interfaces (Schutt et al., 1993). Third, the barbed-end edge of the actin monomer, defined by the binding site for gelsolin (McLaughlin et al., 1993), lies at an end of the ribbon. Fourth, the interface between actin monomers in the ribbon makes use of the same site in subdomains 2 and 4 of actin that is used in binding DNase I (Kabsch et al., 1990; Schutt et al., 1993; Rozycki et al., 1994) (see also Fig. 1), a protein with known actin-depolymerizing activity. Finally, the radial position of Cys-374 in the ribbon, 21.3 Å for Sγ, is consistent with placement of this residue in the filament by undecagold labeling and cryo-electron microscopy (Milligan et al., 1990), after accounting both for the length of the cross-link between the sulfhydryl and the undecagold moiety and for the necessary unfolding of the partially buried Cys-374.

The actin ribbon devoid of profilin (R-actin) is not itself a model for the helical actin filament (H-actin), but we believe it could be transformed into H-actin by some combination of interdomain movements in the actin molecule. The actin:actin ribbon contact would correspond to the one-start helical contact formed between each actin molecule N and its N−1 and N+1 neighbors, whereas profilin molecules would block formation of the two-start (N → N-2 and N → N+2) helical contacts (Cedergren-Zeppezauer et al., 1994). Thus, removal of profilin would permit both the formation of the two-start contacts and the pivoting between actin subdomains necessary to prevent ribbon contacts from breaking during the 13° twist and 8.3 Å shortening per monomer (Schutt et al., 1994) as the ribbon transforms to the helix. Hexokinase, which has a polypeptide fold similar to actin (Kabsch et al., 1990), may serve as a paradigm for structural transitions in actin, because crystallographically observed subdomain movements in actin (Fig. 2) occur at hinge points that are homologous to those used by hexokinase in binding glucose and ATP (Schutt et al., 1993).

A model for F-actin has been proposed (Holmes et al., 1990), based on a fit of the α-actin crystal structure (Kabsch, et al., 1990) to x-ray fiber diffraction data (Popp et al., 1987) and refinement of subdomain positions in actin to achieve an improved fit to the x-ray pattern (Lorenz et al., 1993). Although this model has several features in common with the ribbon, such as the locations of the filament barbed end and

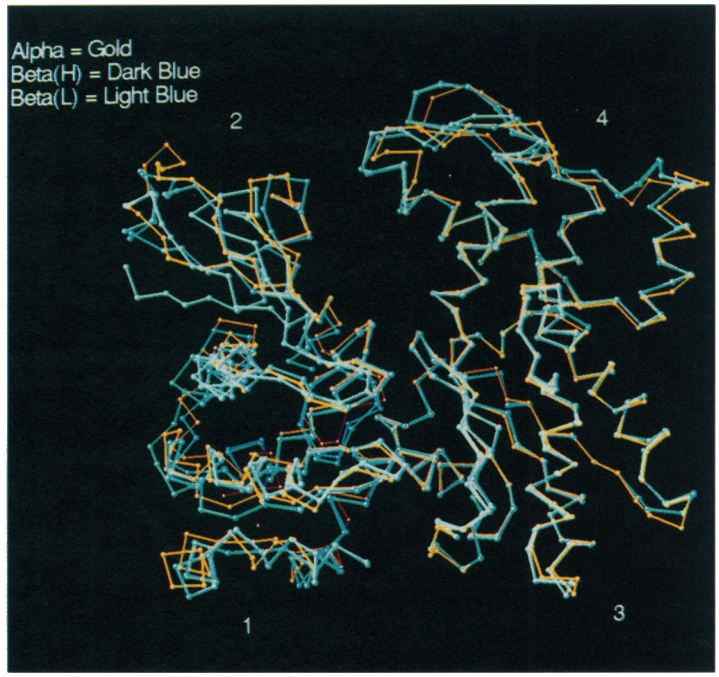

FIGURE 2 Comparison of the structural states of actin. All actin structures were superimposed at residues 135–182 and 263–335 using X-PLOR (Brünger, 1992). Cα traces are shown of α-actin (Kabsch et al., 1990) in gold, β-actin in the tight state (high salt concentration, or beta(H)) in dark blue, and β-actin in the expanded state (low salt concentration, or beta (L)) in light blue.

Cys-374, the F-actin model does not use the same one-start helical contacts found in the ribbon, and the two structures are incompatible with each other (Mendelson and Morris, 1994).

RIBBON-HELIX TRANSITIONS

It is possible that the profilin:actin ribbon not only is structurally related to the filament, but may undergo a transition to it under physiological conditions. The most direct evidence for this is the previously described change in the diffraction pattern of crystals to a fiber-like state (Schutt et al., 1989). However, ribbon-helix transitions are also supported by the increasing evidence (Pantaloni and Carlier, 1993) that profilin plays an active role in actin filament assembly.

The ability of profilin to form extensive contacts with two actin molecules in profilin:actin crystals (Schutt et al., 1993) is consistent with evidence that actin filaments can grow by adding profilin:actin heterodimers to filament barbed ends (Pring et al., 1992; Pantaloni and Carlier, 1993). In actin filament assembly, ribbon-to-helix transitions might be coupled to the dissociation of profilin, allowing actin to accommodate both ribbon and helix lattices in the same filament (Cedergren-Zeppezauer et al., 1994). ATP hydrolysis lags behind monomer addition during filament assembly, so that filaments have ATP "caps" at growing ends whereas monomers at the interior of filaments contain ADP (Carlier and Pantaloni, 1986). Short stretches of profilin:actin ribbons at filament barbed ends would offer a means of coupling dissociation of profilin to ATP hydrolysis during F-actin assembly. Furthermore, a "switch" at the N terminus of profilin is located at the smaller of the two profilin:actin interfaces in the profilin:actin ribbon, adjacent to the poly(L-proline) binding site (Fig. 3). This site is positioned to allow simultaneous interactions between profilin and actin and between profilin and proline-containing peptides (Cedergren-Zeppezauer et al., 1994). The N-terminal switch could affect the binding of either actin, poly(L-proline), or both to profilin.

ACTOMYOSIN AND FORCE GENERATION

Ribbon-to-helix transitions have more specific application in a new theory of force generation (Schutt and Lindberg, 1992). The packing of actin:ADP~P_i monomers into metastable ribbons would allow for a direct coupling of phosphate release to force generation when the contraction to the actin:ADP helix takes place (Fig. 4). Myosin cross-bridges have two functions in this model: first, to initiate the transition from the H-actin state to the R-actin state, and second, to bear the tension produced by F-actin as it returns from the R-state to the H-state. The binding of myosin S1 in the ADP~P_i state to F-actin releases P_i from myosin and initiates the formation of ribbon segments by transforming adjacent actin monomers into a state that facilitates the cooperative uptake of ATP by successive actin monomers. ATP hydrolysis to ADP~P_i on actin leaves actin in a metastable ribbon state. Exchange of ADP for ATP by myosin S1 accompanies its release from F-actin, allowing both the release of P_i from F-actin and a transition from R-actin to H-actin that generates force. In this way, the elongated, high energy actin ribbons extend themselves towards the center of the sarcomere. Subsequent hydrolysis of ATP on myosin primes the S1 head for the next cycle.

The force generated in each cycle would be on the order of 100 pN (Schutt and Lindberg, 1993). By contrast, theories of motility in which actin filaments passively serve to transmit force require that myosin "motors" produce forces in the 1–2 pN range. The main difference in these two viewpoints is that, in the actin-based model, a small number of actin

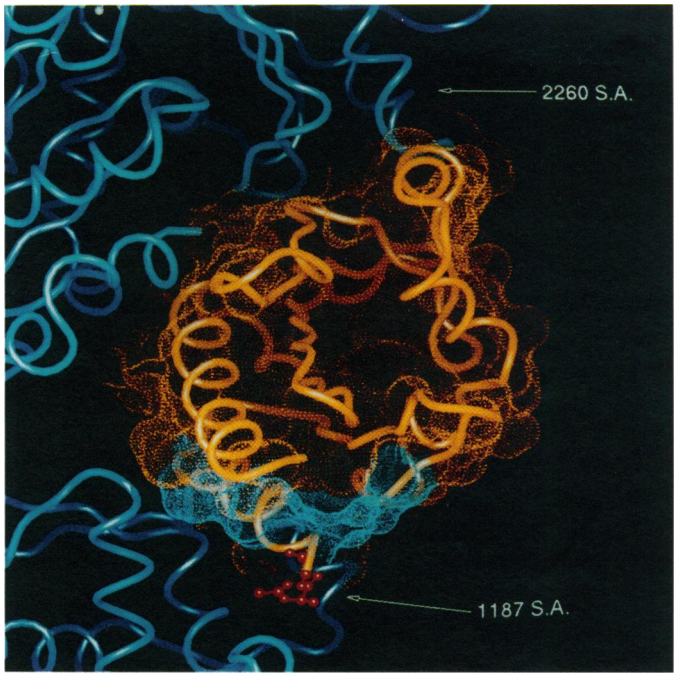

FIGURE 3 Proximity of poly(L-proline) binding site of profilin to interfaces with actin. A portion of the profilin:actin ribbon in Fig. 1 was enlarged to show details of profilin (from the right side of the 2_1-screw axis) and neighboring actin molecules. Proteins are in same colors as in Fig. 1. Profilin is shown as both a main-chain trace and a solvent-accessible surface, with the hydrophobic poly(L-proline) binding surface (Björkegren et al., 1993; Archer et al., 1994; Metzler et al., 1994) in blue. Residues constituting the N-terminal "switch" (Cedergren-Zeppezauer et al., 1994) are shown in red. Buried solvent-accessible surface at the two profilin:actin interfaces was calculated as for Fig. 1.

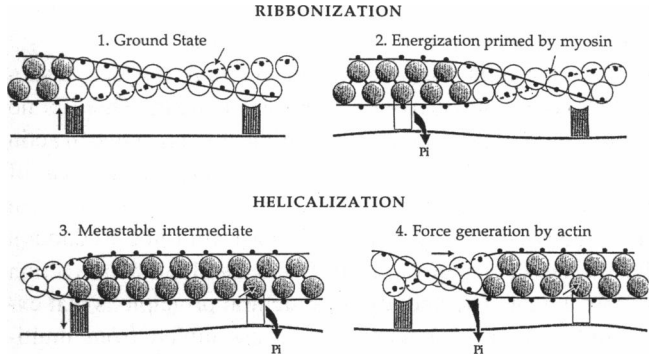

FIGURE 4 The actin powerstroke model of muscle contraction. The ground state consists of helical F-actin (H-actin) in the ADP state detached from myosin S1 in the ADP~P_i state 1. Attachment of myosin S1 to F-actin leads to release of P_i from S1 and transition of F-actin to a pre-ribbon state, which facilitates uptake of ATP by F-actin 2. Hydrolysis of ATP to ADP~P_i by actin leaves a metastable ribbon state, and exchange of ADP for ATP by myosin S1 accompanies its release from F-actin 3, allowing release of P_i from F-actin and an R → H transition that generates force 4. Subsequent hydrolysis of ATP on myosin primes the S1 head for the next cycle. From (Schutt and Lindberg, 1992).

molecules in a filament is generating a strong force at any instant, whereas for myosin-based models, many myosin motors, each generating a considerably weaker force acting over a longer distance, pull simultaneously on a passive actin filament. The Gibbs free energy consumed in moving a fixed load, supplied by the hydrolysis of ATP, is equal to the integrated force times the distance moved, and can be the same for the two models.

DETAILS OF THE ACTIN POWERSTROKE

Actin molecules in the R-actin state are related to those in the H-actin state by a twist of 13° and a positive length change of 8.3 Å, from 27.5 to 35.8 Å per monomer. Thirteen actin monomers (with 12 intermonomer bonds) in the R-state comprise a ribbon segment that spans a distance of 429 Å (12 × 35.8 Å). This is the known repeat distance along the myosin thick filament in the intact muscle sarcomere. It is the distance at which a myosin head in the next layer of myosin heads has the same orientation relative to the ribbon segment as the head that initiated the ribbon segment under consideration. Thus, in this one-dimensional model, the myosin head is automatically in position to bind to the last monomer (i.e., the 13th one) of the extending ribbon segment with the same angle of attachment as the other attached heads. The binding of this head initiates the formation of the next ribbon segment along the actin filament (Fig. 4).

The myosin head that initiated the H → R transformation of the segment is detached when the R → H transition front (or "helicalization front") in the preceding segment reaches it. At this stage in the actin powerstroke, the segment of ribbon on its M-line side can begin to generate force. Actin monomers in the metastable R-state at the initiating end of the segment now return to the stable H-state. Force is generated in the actin filament as each monomer undergoes the R → H transition. The force-generating stage is complete when the last monomer "twists off" the myosin head that is bearing the force generated by the segment. The actomyosin link thus dissociates as the helicalization front passes through it into the next ribbon segment. The detaching myosin head binds ATP, hydrolyzes it, and waits in readiness for the next properly oriented R-actin monomer to arrive.

Actin monomers in the H-state are bound to a tropomyosin filament. As the conversion from the H-state to the R-state proceeds, the connection of each actin subunit to its associated tropomyosin filament is broken. This allows the transmission to the I-band, and also the summation of the independent forces generated by different helicalizing segments along the actin filament. As the return to the H-state takes place, each actin monomer rebinds in succession to its associated tropomyosin filament. The force is developed at the leading edge of the helicalization front that is advancing through the previously ribbonized segment. The anchored ribbon thus pulls the Z-disk towards the center of the sarcomere as it shortens. A contracting ribbon segment develops force between two points of attachment: one where the tropomyosin filament is attached to helical regions at one end of an actin segment in which R-actin monomers are returning to the H-actin state, and the other where it is attached to the

thick myosin filament via the next properly positioned myosin head 429 Å further along the thick filament.

Thus, the actin powerstroke model retains the idea of specific binding sites between actin and myosin. There is no need for a "rolling" interface (multiple bonds) between actin and myosin, nor large conformational changes in any part of the myosin molecule, nor active tilting of myosin heads in the direction of sliding. Instead, the basis for force production lies in length changes in actin filaments along the direction of movement. The underlying structural principle is that extendible protein polymers can be assembled from multidomained subunits. Length changes can be brought about within such polymers without breakage of the intermonomer contacts as long as rotations about hinges between domains in the monomer are possible, as is observed in the actin monomer.

Accordingly, length changes in actin could occur in accordance with the principle of conserved contacts and variable linkages, and a mechanism can be devised in which the rate of the transitions between R- and H-actin depends upon the controlled release of the products of ATP hydrolysis from actin. The key is that the elongated form of actin can remain in a metastable state until "triggered" to contract to the ground state, stabilized by an additional class of bonds.

It is well established that the troponin-tropomyosin system confers calcium-regulation on the relaxation-contraction cycle. Our model retains some of the aspects of the original steric blocking model (Huxley, 1973), which implies that myosin and tropomyosin are antagonistic in their binding to actin filaments. At the end of each actin powerstroke, mechanical events at the helicalization front (where tropomyosin is rebinding) control the detachment of tension-bearing myosin cross-bridges from the actin filaments in the presence of ATP.

The proposed function of tropomyosin to attach and detach from contractile actin filaments enables it to control the transmittal of the developed forces to the ends of the sarcomere as described previously (Schutt and Lindberg, 1992). When a muscle is activated, the binding of calcium by troponin engages the tropomyosin "transmission." Correspondingly, the removal of calcium leads to cessation of helicalization and subsequent relaxation of the muscle fiber. The physiologically *relaxed* state would be characterized by a greater proportion of the ribbon state compared with the *contracting* state, as can be inferred from the x-ray diffraction patterns (Schutt and Lindberg, 1992), and is thus a molecular *high energy* state in contradistinction to its physiological designation.

A premise of the tight coupling hypothesis of free energy transduction in biological systems is that work is produced at the point where the products of nucleotide hydrolysis are released into solution (Eisenberg and Hill, 1985). In the actin powerstroke model, product release occurs at two places (Fig. 4): 1) from myosin heads when they bind to actin to initiate the H $\rightarrow$ R transition in actin subunits (the initiation step), and 2) from individual R-actin monomers shortening the segment as they revert to the H-actin form and rebind to tropomyosin (the work-producing step). Phosphate release from actin is stimulated by the rebinding of tropomyosin.

In vitro motility assays yield data suggesting that tension proportional to actomyosin overlap can be achieved without tropomyosin (VanBuren et al., 1994). Although these experiments could be interpreted as contradictions to a central requirement of the actin powerstroke model, they can in fact be rationalized in terms of the mechanism presented above, whereby myosin heads provide attachment points to thick filaments for the force-producing actin segments, and the number of heads determines the maximum tension that can be borne. Finally, our results are not inconsistent with electron microscopy (Sosa et al., 1994), because on the average only two ribbon-to-helix transition fronts per actin filament are needed to generate the observed tension (200 pN per actin filament at τ_{max}). This would require an increase in filament length of only 0.02 μm, a value well within the experimental error of the measurements of Sosa and co-workers.

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DISCUSSION

Session Chairperson: Yale E. Goldman
Scribe: Perry Sun

G. POLLACK: Your work predicts two lengths of actin filaments, a short one and a long one. We have some evidence from studies in intact sarcomeres that seems to be consistent with that possibility [published in Jonas et al. 1993]. Although we get 38 nm for the fine I-band periodicity, sometimes we also get 43 nm. We believe these measurements are correct from careful calibration. We don't know the circumstances under which these spacings are possible, but perhaps the dual spacing could imply consistency with your idea that the actin filament could have two lengths.

SCHUTT: Thank you.

E. REISLER: One of the differences that you are pointing out between the different models is in the shape and position of subdomain 2. Are you assigning a functional role to the motions of subdomain 2 in your model?

SCHUTT: In our model, subdomain 2 is a critical part of the ribbon interface, so it can flex as the filament goes into the helical state. But I want to mention the principle of conserved contacts and variable linkages discovered by Steve Harrison and others in virus crystallography. Viruses also expand and contract, and they are polymeric assemblies (they are icosahedral). They do so by conserved protein-protein interactions with hinge areas. So viruses can expand and contract without breaking these types of contacts. So I think subdomain 2 is critical in this transition. In response to G. Pollack's question: if the role of myosin in muscle contraction is to bind to actin, use its free energy of hydrolysis to open up actin to bind ATP, and that ATP hydrolysis on actin leads to force generation, and as you go from monomer to monomer, pulling against tropomyosin, then you predict that the force generated by an actin monomer is roughly 100 pN; that is, 8.3 · 10e-23 J (energy of hydrolysis of one ATP molecule) divided by 8 Å the ribbon-to-helix transition in one monomer. Therefore, in an isotonic contraction, the force observed during filament sliding can be generated by just one actin molecule at a time during transition from the ribbon to the helix. You can think of this as waves moving along, much like Oosawa did many years ago. Oosawa had the insight that actin could do this, but Oosawa's models could never overcome all of the objections posed by A. F. Huxley's analysis from Huxley and Simmons' data. So I think that a hybrid filament theory is really necessary. Actually there is very little change going on in actin even though it is generating large forces; that is because the force is generated over short distances. So attempts to see this by spectroscopy will probably fail, because not much is happening. And of course the heads don't have to rotate very much. Why? Because they, in a sense, wait in readiness for the actin ribbon segment to come along and bind to the head. So there are two ATPases, the myosin ATPase and the actin ATPase, and they are regulated by the dynamics of the filament lattice.

K. HOLMES: In your comparison to the two models, I must say a few words. First, when we did our test of the actin monomer against the diffraction pattern we in fact tried out all possible conformations in 10° intervals, and then refined the three most likely candidates. So I think we have tried out all possible orientations and since your orientation has been tried it won't fit the diffraction pattern. If you give me your coordinates I'll try it, but my guess is that it won't. Second, I would say that the position of the myosin binding site on the outer surface of subdomain 1 is well established by biochemists. You don't seem to put the outer surface of subdomain 1 in the right place. Isn't that a severe problem?

SCHUTT: To answer your first question, the motions we have seen in subdomain 2 are still with the ribbon intact in the crystal. I think there are further motions possible in the actin molecules, so I don't think you have tested all possibilities. There are serious problems with the Holmes model having to do with the way they stabilize the two strands of the double-stranded helix. This is the famous hydrophobic plug. We see no evidence for any movement of this hydrophobic plug away from subdomain 4 to form such a plug, and we find this argument highly specious.